August 12, 1949

Dr. Joshua Lederberg
Department of Genetics
University of Wisconsin
Madison, Wisconsin

Dear Dr. Lederberg:

I have started the crosses, and I felt that you would be interested to hear the results thus far obtained. As you shall see things have not turned out to be as simple as was expected. The crosses were all done in EMS lactose with B1, and no streptomycin. The genotype of the parents was BM lac plus T1S by TLB1 lac negative T1R with streptomycin resistance and dependence in either or both stocks, depending on the specific cross involved. When resistant was crossed with resistant all the prototrophs were streptomycin resistant, indicating that the genes involved were alleles. The yields were surprisingly low (most crosses on 10 plates) and the scoring for the lactose character somewhat difficult because of the mucoid character in the resistant stocks which was segregated in the progeny.

The data of lac and the T1 segregation are below.

B^-M^- lac+ V_1^S "S" X $TL^-B_1^-$ lac- V_1^R "S"

	lac+ V_1^S	lac- V_1^S	lac+ V_1^R	lac- V_1^R
S-1 X S-3	51	36	8	19
S-2 X S3	6	4	0	1
S-20 X S26	16	15	0	6
S-20 X S25	19	15	4	10
S-19 X S25	18	14	1	5
S-19 X S26	25	15	2	5
S-21 X S25	15	10	1	2
S-21 X S26	19	13	1	4
Total 365	46%	34.8%	4.7%	14.5%

Going under the hypothesis that these were alleles segregating and having no effect upon the lactose Tl segregation I took the liberty of summating these figures and calculated the recombination percentages from these. As is obvious the segregation of lac and tl has in some way been altered. The percent recombination, both for the singles and the triples, is in accord with your data. The gross excess of the one parent and the deficiency of the other, plus the consistency of the da make data it appears as if some semilethal was linked to Tl or that the population dynamics is applying a strong selective pressure against the lac- Tlr class.

The next set of crosses were between resistants and dependants. Here again all of the prototrophs were streptomycin resistant, once again indicating that a single "locus " was involved.

(S19) $B^- M^-$ lac+ V_1^S "S" X $T^- L^- B_1^-$ lac(+) V_1^R "Sd" (Sd1)

lac+ V_1^S	lac- V_1^S	lac+ V_1^R	lac- V_1^R
202	51	9	3

(Sd14) $B^- M^-$ lac+ V_1^S "Sd" X $T^- L^- B_1^-$ lac- V_1^R "S" sd25

4	10	0	4

In order to equate everything the procedure outlined below was put into effect.

sd13 = Sd1 = Sd14 = Sd2 (sd were all biologically different in their utilization of streptobiosamine.)

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S26 = S19 = S25 = S20 = S21

| |

outcross outcross

At this point I tended to believethat the so called streptomycin resistant was partially dependant (qualitative observations on the growth of resistants in the presence and absence of streptomycin) and that the segregation above was only the extreme of that previously mentioned.

The crosses of Sd by Sd yielded no prototrophs and at this point Dr. Demerec left for his vacation happy and secure in the knowledge that he was dealing with a single complex gene, the queer lac T1 segregation not interesting him. The prototrophs from the outcross were to my great amazement all streptomycin resistant. Adequate controls on the medium used and the parental reactions were run. The lac T1 segregation was consistant again.

58-161 X T⁻L⁻B₁⁻ lac- V_1^R "S"

Lac- V_1^R	Lac- V_1^S	Lac+ V_1^R	Lac+ V_1^S
15	46	11	225

possibly somewhat biased up as the lab assistants tended to pick not the large + colonies

W595 X B⁻M⁻ lac+ V_1^S "S"

18	69	10	162	(my data)
6%	28%	4%	61%	

The mucoid character again interfered with the lac scoring.
Several hypothses came into mind:

1- There may be many genes involved or just two, one at each end, but these are supposedly single steps mutations
2-prototrophs were heterozygotes but this is invalidated by even the extremely small % lac-
3-we are dealing with an extra genic factor or one coupled with a gene

Though somewhat wild I've been thinking strongly along this latter line as it might also explain some queer data Bertani has been obtaining with reversions from dependance. The protocol I've decided upon for the remainder of my stay is as follows:

1- Repeat the outcrosses(not another Ravin)
2-Cross the wild types as a control of their genetic constitution especially in regard to the lac T1
3-Cross resistant by dependant on streptomycin medium to determine if I can recover both types or once again only resistants
4- Outcross the dependantsand proceed with an analysis

of the type mentioned above (these latter two showing nothing much if they give both types but indicatory if only one occurs)

5- If the original data is reproduceable pick a sample of the prototrophs and transfer them daily testing for resistanceas theirs might (cytoplasmic interpretation)be two types if the factor "c" is gene reproduced and hence could be diluted out by serial transfer from those only phenotypically resistant.

6- Any suggestions will be appreciated

Of course the most efficient approach would be to put a resistant through a heterozygote. Frankly I don't think that they will be able to follow through here and I must wait for Demerec's return before going into such and of course your okay on my follow through at Wisconsin.

I ran that experiment with Adams . SW- 87 was streaked free of phage and grown up in nutrient broth. It was subcultured into broth containg the usual sugars, making a faintly turbid suspension, and 10^7 phage particles added. Clearing occured in the lactose and the galactose tube after 35 minutes (single burst?) and an increase in turbidity in the nutrient broth and dextrose.This behavior was typical of the parent (SW-13) and indicates that it is a direct action of the sugars with no necessity of polysaccharide formation. Absorbtion experiments are in order as soon as I can obtain a reliable assay on the phage stock (produces clearer plaques in Hershey agar. Finding some difficulty in the disposal of contaminated material and nott wanting to further impose on Adams I've let the matter lie.

My best regards to you and Mrs. Lederberg.

Sincerely,

Norton

Norton Zinder